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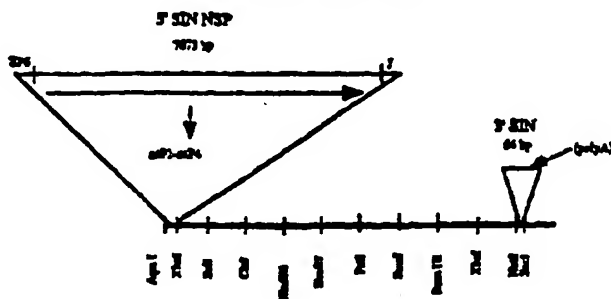
(54) Title: **RECOMBINANT ALPHAVIRUS VECTORS**

(57) Abstract

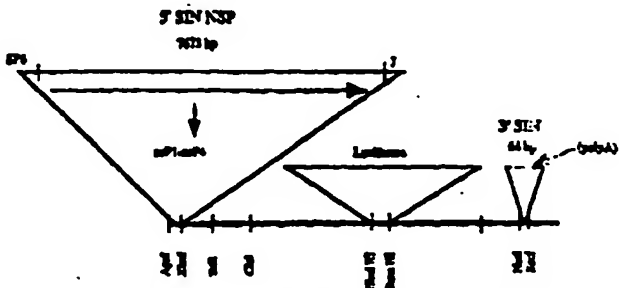
The present invention provides composition and methods for utilizing recombinant alphavirus vectors.

SINDBIS EXPRESSION VECTORS

SINDBIS Basic Vector



SINDBIS-Incubator



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WHAT IS CLAIMED

1. An alphavirus vector construct, comprising a 5' promoter which is capable of initiating the synthesis of viral RNA *in vitro* from cDNA, a 5' sequence which is capable of initiating transcription of an alphavirus, a nucleotide sequence encoding alphavirus non-structural proteins, a viral junction region which has been inactivated such that viral transcription of the subgenomic fragment is prevented, and an alphavirus RNA polymerase recognition sequence.
2. An alphavirus vector construct, comprising a 5' promoter which is capable of initiating the synthesis of viral RNA *in vitro* from cDNA, a 5' sequence which is capable of initiating transcription of an alphavirus, a nucleotide sequence encoding alphavirus non-structural proteins, a viral junction region which has been modified such that viral transcription of the subgenomic fragment is reduced, and an alphavirus RNA polymerase recognition sequence.
3. An alphavirus vector construct, comprising a 5' promoter which is capable of initiating the synthesis of viral RNA *in vitro* from cDNA, a 5' sequence which is capable of initiating transcription of an alphavirus, a nucleotide sequence encoding alphavirus non-structural proteins, a first viral junction region which has been inactivated such that viral transcription of the subgenomic fragment is prevented, a second viral junction region which has been modified such that viral transcription of the subgenomic fragment is reduced, and an alphavirus RNA polymerase recognition sequence.
4. An alphavirus cDNA vector construct comprising a 5' promoter which is capable of initiating the synthesis of viral RNA from cDNA followed by a 5' sequence which is capable of initiating transcription of an alphavirus, a nucleotide sequence encoding alphavirus non-structural proteins, a viral junction region which is either active or which has been inactivated such that viral transcription of the subgenomic fragment is prevented, an alphavirus RNA polymerase recognition sequence, and a 3' sequence which controls transcription termination.
5. An alphavirus cDNA vector construct comprising a 5' promoter which is capable of initiating the synthesis of viral RNA from cDNA followed by a 5' sequence which is capable of initiating transcription of an alphavirus, a nucleotide sequence encoding alphavirus non-structural proteins, a viral junction region which has been modified such that viral transcription of the subgenomic fragment is reduced, an alphavirus RNA polymerase recognition sequence, and a 3' sequence which controls transcription termination.

6. An alphavirus cDNA vector construct comprising a promoter which is capable of initiating the synthesis of viral RNA from cDNA followed by a 5' sequence which is capable of initiating transcription of an alphavirus, a nucleotide sequence encoding alphavirus non-structural proteins, a first viral junction region which has been inactivated such that viral transcription of the subgenomic fragment is prevented, followed by a second viral junction region which has been modified such that viral transcription of the subgenomic fragment is reduced, an alphavirus RNA polymerase recognition sequence, and a 3' sequence which controls transcription termination.
7. The vector construct according to any one of claims 1 to 6, further comprising a polyadenylation sequence.
8. The vector according to any one of claims 1-6 wherein said alphavirus is selected from the group consisting of Aura, Fort Morgan, Venezuelan Equine Encephalitis, Ross River, Semliki Forest and Mayaro.
9. The vector according to any one of claims 1-6 wherein said alphavirus is Sindbis virus.
10. The vector according to any one of claims 1-6 wherein said vector construct contains a selected heterologous sequence.
11. The vector of claim 10 wherein said vector construct contains a heterologous nucleotide sequence of greater than 100 bases.
12. The vector of claim 10 wherein said vector construct contains a heterologous nucleotide sequence of greater than 8 kb
13. The vector of claim 10 wherein said selected heterologous sequence is a sequence encoding a protein selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, γ -IFN, G-CSF, and GM-CSF.
14. The vector of claim 10 wherein said selected heterologous sequence is obtained from a virus selected from the group consisting of influenza virus, respiratory syncytial virus, HPV, HBV, HCV, EBV, HIV, HSV, FeLV, FIV, Hantavirus, HTLV I, HTLV II, and CMV.

15. The vector of claim 10 wherein said selected heterologous sequence is an antisense, a non-coding sense sequence or ribozyme sequence.

16. The vector of claim 15 wherein said antisense or noncoding sense sequence is selected from the group consisting of sequences which are complementary to influenza virus, respiratory syncytial virus, HPV, HBV, HCV, EBV, HIV, HSV and CMV sequences.

17. The vector according to any one of claims 1-6 wherein said vector contains no alphavirus structural proteins genes.

18. The vector of claim 1, 2, 4 or 5 wherein a selected heterologous sequence is located downstream from said viral junction region.

19. The vector of claim 3 or 6 wherein a selected heterologous sequence is located downstream from said second viral junction region.

20. The vector of claim 18, further comprising a polylinker located subsequent to said viral junction region.

21. The vector of claim 18 wherein said polylinker does not contain a wild-type alphavirus restriction endonuclease recognition sequence.

22. The vector of claim 10 wherein said selected heterologous sequence is located within a nucleotide sequence encoding alphavirus non-structural proteins.

23. The vector of claim 1 or 4 wherein said modified viral junction region consists of the nucleotide sequence as shown in Figure 3, from nucleotide number 7579, to nucleotide 7597.

24. The vector of claim 3 or 6, further comprising an adenovirus E3 gene or CMV H301 gene located downstream from said second viral junction region.

25. The vector of claim 3 and 6, further comprising a retroviral packaging sequence located between said first viral junction region and said second viral junction region.

15. The vector of claim 10 wherein said selected heterologous sequence is an antisense, a non-coding sense sequence or ribozyme sequence.

16. The vector of claim 15 wherein said antisense or noncoding sense sequence is selected from the group consisting of sequences which are complementary to influenza virus, respiratory syncytial virus, HPV, HBV, HCV, EBV, HIV, HSV and CMV sequences.

17. The vector according to any one of claims 1-6 wherein said vector contains no alphavirus structural proteins genes.

18. The vector of claim 1, 2, 4 or 5 wherein a selected heterologous sequence is located downstream from said viral junction region.

19. The vector of claim 3 or 6 wherein a selected heterologous sequence is located downstream from said second viral junction region.

20. The vector of claim 18, further comprising a polylinker located subsequent to said viral junction region.

21. The vector of claim 18 wherein said polylinker does not contain a wild-type alphavirus restriction endonuclease recognition sequence.

22. The vector of claim 10 wherein said selected heterologous sequence is located within a nucleotide sequence encoding alphavirus non-structural proteins.

23. The vector of claim 1 or 4 wherein said modified viral junction region consists of the nucleotide sequence as shown in Figure 3, from nucleotide number 7579, to nucleotide 7597.

24. The vector of claim 3 or 6, further comprising an adenovirus E3 gene or CMV H301 gene located downstream from said second viral junction region.

25. The vector of claim 3 and 6, further comprising a retroviral packaging sequence located between said first viral junction region and said second viral junction region.

26. An isolated recombinant alphavirus vector which does not contain a functional viral junction region.

27. An isolated recombinant alphavirus vector which produces reduced viral transcription of the subgenomic fragment.

28. An alphavirus structural protein expression cassette, comprising a promoter and one or more alphavirus structural protein genes, said promoter being capable of directing the expression of said alphavirus structural protein.

29. The expression cassette of claim 28 wherein said alphavirus structural protein is derived from an alphavirus selected from the group consisting of Aura, Fort Morgan, Venezuelan Equine Encephalitis, Ross River, Semliki Forest, Sindbis, and Mayaro viruses.

30. The expression cassette of claim 28 wherein said alphavirus structural protein is the alphavirus capsid protein.

31. The expression cassette of claim 28 wherein said alphavirus structural protein is selected from the group consisting of alphavirus structural proteins 6K, E3, E2, and E1.

32. An alphavirus structural protein expression cassette, comprising a promoter, one or more alphavirus structural proteins, and a heterologous ligand sequence, said promoter being capable of directing the expression of said alphavirus structural proteins and said heterologous sequence.

33. The expression cassette according to any one of claims 28 to 32 wherein said promoter is selected from the group consisting of metallothionein, Drosophila actin 5C distal, SV40, heat shock protein 65, heat shock protein 70, Py, RSV, BK, JC, MuLV, MMTV, alphavirus junction region, CMV and VA1RNA.

34. A recombinant alphavirus particle which, upon introduction into a BHK cell, produces an infected cell which is viable at least 24 hours after infection.

35. A recombinant alphavirus particle which, upon introduction into a BHK cell, produces an infected cell which is viable at least 24 hours after infection, said particle also carrying a vector construct which directs the expression of at least one antigen or modified form thereof in target cells infected with the alphavirus particle, said antigen or modified form thereof being capable of stimulating an immune response within an animal.

36. The recombinant particle of claim 35 wherein the expressed antigen elicits a cell-mediated immune response.

37. The recombinant alphavirus particle of claim 35 wherein the expressed antigen elicits an HLA class I- restricted immune response.

38. The recombinant alphavirus of claim 35 wherein the expressed antigen further elicits an HLA Class II-restricted immune response.

39. A recombinant alphavirus particle which carries a vector construct capable of directing the expression of a palliative in cells infected with the alphavirus particle, said palliative being capable of inhibiting a function necessary for the pathogenicity of a pathogenic agent.

40. The recombinant alphavirus particle of claim 39 wherein the pathogenic agent is a cancerous cell or cancer-promoting growth factor.

41. The recombinant alphavirus particle of claim 39 which directs the expression of a toxic palliative in infected target cells in response to the presence in said cells of an entity associated with the pathogenic agent.

42. The recombinant alphavirus particle of claim 39 wherein the palliative is capable of selectively inhibiting the expression of a pathogenic gene.

43. The recombinant alphavirus particle of claim 39 wherein the palliative is capable of inhibiting the activity of a protein produced by the pathogenic agent.

44. The recombinant alphavirus particle of claim 39 wherein the palliative comprises antisense RNA complementary to RNA sequences necessary for pathogenicity.

45. The recombinant alphavirus particle of claim 39 wherein the palliative comprises sense RNA complementary to RNA sequences necessary for pathogenicity.

46. The recombinant alphavirus particle of claim 39 wherein the palliative comprises a defective structural protein of a pathogenic agent, said protein being capable of inhibiting assembly of the pathogenic agent.

47. The recombinant alphavirus particle of claim 39 which directs the expression of a gene product capable of activating an otherwise inactive precursor into an active inhibitor of the pathogenic agent.

48. The recombinant alphavirus particle of claim 39 which directs the expression of the herpes thymidine kinase gene product.

49. The recombinant alphavirus particle of claim 39 which directs the expression of an RNA molecule which functions as a ribozyme specific for a RNA molecule required for pathogenesis.

50. A recombinant alphavirus particle which directs the expression of a gene capable of suppressing one or more elements of the immune system in target cells infected with said alphavirus particle.

51. A mammalian cell infected with a recombinant alphavirus particle according to any one of claims 34 to 50.

52. A method of stimulating an immune response to an antigen, comprising infecting susceptible target cells with a recombinant alphavirus particle which directs the expression of at least one antigen or modified form thereof in target cells infected with the alphavirus, said antigen or modified form thereof being capable of stimulating an immune response within an animal.

53. The method of claim 52 wherein the target cells are infected *in vivo*.

54. The method of claim 52 wherein the expressed antigen elicits an HLA class I-restricted immune response.

55. The method of claim 52 wherein the expressed antigen further elicits an HLA Class II-restricted immune response.

56. The method of claim 52, including, prior or subsequent to the step of infecting target cells, introducing into target cells a nucleic acid molecule which encodes either Class I or Class II MHC protein, or combinations thereof, or a protein selected from the group consisting of CD3, ICAM-1, LFA-3 or analogues thereof.

57. A method of inhibiting a pathogenic agent, comprising infecting susceptible target cells with a recombinant alphavirus particle which directs the expression of a palliative in cells infected with the alphavirus particle, said palliative being capable of inhibiting a function of a pathogenic agent necessary for pathogenicity.

58. The method of claim 57 wherein the pathogenic agent is a cancerous cell or cancer-promoting growth factor.

59. The method of claim 57 wherein the recombinant alphavirus particle directs the expression of a toxic palliative in infected target cells in response to the presence in said cells of an entity associated with the pathogenic agent.

60. The method of claim 57 wherein the palliative comprises antisense RNA complementary to RNA sequences necessary for pathogenicity.

61. The method of claim 57 wherein the palliative comprises sense RNA complementary to RNA sequences necessary for pathogenicity.

62. The method of claim 57 wherein the palliative comprises a defective structural protein of a pathogenic agent, said protein being capable of inhibiting assembly of the pathogenic agent.

63. The method of claim 57 wherein the alphavirus particle directs the expression of a gene product capable of activating an otherwise inactive precursor into an active inhibitor of the pathogenic agent.

64. The method of claim 57 wherein the alphavirus particle directs the expression of the herpes thymidine kinase gene product.

65. The method of claim 57 wherein the alphavirus particle directs the expression of an RNA molecule which functions as a ribozyme specific for a RNA molecule required for pathogenesis.

66. A method of inhibiting the binding of an agent to a receptor associated with a cell, comprising infecting susceptible target cells with a recombinant alphavirus particle which directs the expression of a blocking element in cells infected with said alphavirus particle, said blocking element being capable of binding to either a receptor or an agent such that the receptor/agent interaction is blocked.

67. *Ex vivo* cells infected with a recombinant alphavirus particle according to any of claims 34 to 50.

68. *Ex vivo* cells infected with a recombinant alphavirus particle carrying a retroviral construct.

69. A pharmaceutical composition comprising an alphavirus particle according to any one of claims 34 to 50, in combination with a physiologically acceptable carrier or diluent.

70. A packaging cell line which produces an alphavirus particle.

71. A mammalian packaging cell line which produces an alphavirus particle.

72. A non-mammalian packaging cell line which produces an alphavirus particle.

73. An insect packaging cell line which produces an alphavirus particle.

74. The packaging cell line of claim 73 wherein said insect packaging cell is a mosquito packaging cell.

75. The packaging cell line of claims 70 to 74 wherein the packaging cell line, upon introduction of a vector construct, produces alphavirus particles capable of infecting human cells.

76. The packaging cell line of claims 70 to 74 wherein said packaging cell line produces alphavirus particles in response to one or more factors.

77. The packaging cell line of any one of claims 70 to 74, wherein alphavirus inhibitory protein is not produced.

78. A retroviral-derived producer cell line suitable for packaging and production of an alphavirus vector, comprising an expression cassette which directs the expression of *gag/pol*, an expression cassette which directs the expression of *env*, and alphavirus cDNA vector construct containing a retroviral packaging sequence.

79. A VSV-G derived packaging cell suitable for packaging and production of an alphavirus vector, comprising a stably integrated expression cassette which directs the expression of VSV-G.

80. The packaging cell line of claim 79, further comprising a stably integrated expression cassette which directs the expression of one or more alphavirus structural proteins.

81. An alphavirus producer cell line which is capable of producing recombinant alphavirus particles.

82. The alphavirus producer cell line according to claim 81 wherein said recombinant alphavirus particles are capable of infecting human cells

83. The alphavirus producer cell line according to claim 81 wherein said producer cell line produces recombinant alphavirus particles in response to one or more factors.

84. The alphavirus producer cell line according to claim 81 wherein said producer cell produces alphavirus particles in response to a differentiation state of said producer cell line.

85. A eukaryotic layered vector initiation system, comprising a promoter which is capable of initiating the 5' synthesis of RNA from cDNA, a construct which is capable of autonomous replication in a cell, said construct being capable of expressing a heterologous nucleic acid sequence, and a 3' sequence which controls transcription termination, with the proviso that said eukaryotic layered vector initiation system does not contain sequences which encode alphavirus non-structural proteins.

86. The eukaryotic layered vector initiation system according to claim 95 wherein said construct which is capable of autonomous replication is in opposite orientation to said promoter and said 3' sequence which controls transcription termination.

87. A mammalian cell containing a stably integrated eukaryotic layered vector initiation system according to claim 85 or 86.

88. A eukaryotic layered vector initiation system, comprising a promoter which is capable of initiating the 5' synthesis of RNA from cDNA, a construct which is capable of

autonomous replication in a cell, said construct being capable of expressing a heterologous nucleic acid sequence, and a 3' sequence which controls transcription termination.

89. A eukaryotic layered vector initiation system, comprising a DNA promoter which is capable of initiating the 5' synthesis of RNA from cDNA, a construct which is capable of autonomous replication in a cell, said construct being capable of expressing a heterologous ribonucleic acid sequence, and a 3' DNA sequence which controls transcription termination.

90. The eukaryotic layered vector initiation system according to claim 88 or 89 wherein said construct is an alphavirus vector construct.

91. The eukaryotic layered vector initiation system according to claim 88 or 89 wherein said construct is derived from a virus selected from the group consisting of poliovirus, rhinovirus, coxsackievirus, rubella, yellow fever, HCV, TGEV, IBV, MHV, BCV, parainfluenza virus, mumps virus, measles virus, respiratory syncytial virus, influenza virus, RSV, MoMLV, HIV, HTLV, hepatitis delta virus and Astrovirus.

92. The eukaryotic layered vector initiation system according to claim 88 or 89 wherein said promoter is selected from the group consisting of the MoMLV promoter, metallothionein promoter, glucocorticoid promoter, SV40 promoter, and the CMV promoter.

93. The eukaryotic layered vector initiation system according to claim 88 or 89, further comprising a polyadenylation sequence.

94. The eukaryotic layered vector initiation system according to claim 88 or 89 wherein said selected heterologous sequence is a sequence encoding a protein selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, γ -IFN, G-CSF, and GM-CSF.

95. The eukaryotic layered vector initiation system according to claim 88 or 89 wherein said selected heterologous sequence is obtained from a virus selected from the group consisting of influenza virus, respiratory syncytial virus, HPV, HBV, HCV, EBV, HIV, HSV, FeLV, FIV, Hantavirus, HTLV I, HTLV II, and CMV.

96. The eukaryotic layered vector initiation system according to claim 88 or 89 wherein said selected heterologous sequence is an antisense, a non-coding sense sequence or ribozyme sequence.

97. The eukaryotic layered vector initiation system according to claim 88 or 89 wherein said antisense or noncoding sense sequence is selected from the group consisting of sequences which are complementary to influenza virus, respiratory syncytial virus, HPV, HBV, HCV, EBV, HIV, HSV and CMV sequences.

98. A method for delivering a heterologous nucleic acid sequence to a warm-blooded animal, comprising administering to said warm-blooded animal a eukaryotic layered vector initiation system according to claim 88 to 89.

99. A recombinant alphavirus particle that is resistant to inactivation in serum.

100. A method for titering recombinant alphavirus particles, comprising:
(a) infecting cells according to claim 88 with recombinant alphavirus particles; and
(b) determining the expression of a heterologous nucleic acid sequence, such that said titer may be determined.

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